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IRS2 variants and syndromes of severe insulin resistance

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To the Editor

Numerous lines of experimental evidence implicate IRS2 in insulin signal transduction in key insulin-responsive tissues, and the combination of insulin resistance and pancreatic beta cell failure of homozygous *Irs2* knockout mice is highly reminiscent of both core abnormalities in human type 2 diabetes [1]. *IRS2* is therefore a compelling candidate gene for involvement in prevalent forms of type 2 diabetes and insulin resistance in humans. Nevertheless, genetic studies to date in humans suggest that common polymorphisms found in the *IRS2* coding or promoter regions are not associated with insulin resistance or type 2 diabetes [2, 3, 4]. Furthermore, these studies have revealed few rare non-synonymous variants, none of which appear to have a strong impact on insulin sensitivity [5, 6].

We have assembled a large cohort of patients with severe insulin resistance that is likely to be highly enriched for monogenic disorders of insulin action. All participants gave informed consent, and our investigations were carried out with the approval of the local research

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ethics committee in Cambridge, UK. This strategy has previously been validated, for example, by the finding of a convincing pathogenic mutation in *AKT2* [7], despite a failure to find association between common genetic variation in *AKT2* and metabolic traits [8]. We have now applied this robust approach to *IRS2*.

We sequenced the coding region of *IRS2* in 161 predominantly Europid (n=114) patients with severe insulin resistance. Severe insulin resistance was defined as (1) a fasting insulin level of >150 pmol/l or a peak insulin level on oral glucose tolerance testing of >1,500 pmol/l in those without diabetes and a BMI of <30 kg/m^2, or (2) an exogenous insulin requirement of >3 U/kg/day in those with complete insulin deficiency and a BMI of <30 kg/m^2. Those with partial beta cell decompensation or a BMI of >30kg/m^2 were included at the investigators discretion based on clinical and biochemical features of insulin resistance disproportionate to body weight, determined by reference to sex and BMI-specific 95th percentiles for plasma insulin from more than 500 non-diabetic volunteers.

Eight rare, novel, non-synonymous variants in the *IRS2* gene were identified in eight patients from this cohort (designated as patients SIR1 to SIR8 in Table 1), four of whom are Europids. Wherever possible, family members of probands with non-synonymous variants were studied to look for co-segregation of the genetic variant with insulin resistance or diabetes.

In the case of SIR1 (c.233G>A, p.S78N), four of the five family members studied were found to be heterozygous for this mutation and had insulin levels commensurate with their degree of obesity [9] (fasting insulin levels 45 – 95 pmol/l, BMI 30 −38.8 kg/m^2); hence, there was no clear co-segregation of the mutation with severe insulin resistance in the family (Electronic supplementary material [ESM] Fig. 1), indicating that the S78N change in *IRS2* within this family is either benign, as suggested by Polyphen (http://genetics.bwh.harvard.edu/pph, accessed 4 March 2008)/SIFT (http://blocks.fhcrc.org/sift/SIFT.html, accessed 22 December 2008) analysis, or is not the sole genetic determinant of insulin resistance within the kindred. The location of serine 78 within the Pleckstrin homology domain of the protein (ESM Fig. 2) and its strong evolutionary conservation support the view that a pathogenic role for this protein-altering variant should not be entirely discounted on the basis of these genetic data.

Eight members of the Turkish family of SIR3, with five affected members, were also studied, but only the unaffected father of the proband was found to carry the same heterozygous mutation (c.1570A>G, p.I524V). Thus, this non-synonymous change in *IRS2* convincingly fails to co-segregate with insulin resistance in this family (ESM Fig. 3, ESM Table 1) and is thus most probably benign.

Of the four available relatives of Ashkenazy proband SIR4 (c.2485C>T, p.P829S), three were heterozygous for this variant, two of whom exhibited elevated fasting insulin levels with respect to 500 non-diabetic volunteers (ESM Fig. 4). However, incomplete clinical data precluded a definitive conclusion regarding co-segregation of the mutation with the phenotype in this family. It is of interest that this variant was absent in 185 Ashkenazy controls and that proline 829 is conserved in a diverse range of species, from *Pan troglodytes* (chimpanzee) down to *Xenopus Tropicalis* (Western clawed frog) (ESM Fig. 5).

The non-synonymous variant found in SIR6 (c.2834C>T, p.S945F) was found to be absent in this patient's mother, who presented with a similar but milder syndrome of partial lipodystrophy. Although the father of SIR6 was unavailable for study (ESM Fig. 6), this suggests that this variant does not underlie the observed phenotype.

In a study limited to the parents of SIR8 (ESM Fig. 7), there appeared to be co-segregation of the non-synonymous variant (c.3983A>G, p.H1328R) found in the proband with clinical phenotype, but because this change was also observed twice on sequencing additional controls, this variant was deemed to be benign.

Subsequent to our family studies, we also sequenced the *IRS2* gene in 173 Europid controls (mean fasting insulin 30 pmol/l, mean BMI 27.3 kg/m^2) to assess possible enrichment of non-synonymous variants in the patients with severe insulin resistance relative to insulin-sensitive controls. In the controls (Table 1), we detected four non-synonymous heterozygous variants, one of which (c.3983A>G, p.H1328R) was present in two controls (control 1 and 4), as well as in proband SIR8. These findings suggest that there is no significant difference in the frequency of non-synonymous variants in the *IRS2* gene between the two groups studied, with four non-synonymous variants being found in each group of Europids (4/114 patients vs. 4/173 controls, p=0.54).

Our study has revealed several new non-synonymous variants in the *IRS2* gene in both a cohort of predominately Europid individuals with severe insulin resistance and in insulin-sensitive Europid volunteers. Wherever possible, we have performed co-segregation studies on the available family members of severely insulin-resistant patients, but we have found no clear evidence that any of the non-synonymous variants studied has a fully penetrant pathogenic effect. Given the difficulty involved in performing detailed functional evaluation of each variant, we thought to describe these results in the hope that if others have similar data, aggregate data may provide the impetus required to carry out these additional analyses. However, we cannot rule out the possibility that some of these variants contribute to the phenotype of these patients in combination with environmental or other, unknown, genetic variants. Sequencing of *IRS2* in insulin-sensitive controls demonstrated that there was no enrichment in the number of non-synonymous variants among the cases, but because this study only had 4.85% power (at p=0.05 significance) to detect the modest differences in frequency observed here, much larger studies will be required to elucidate the role of rare *IRS2* variants in disease. To obtain nominal levels of significance (p=0.05), assuming the same proportions of mutation carriers and non-carriers as those we report here, the study would need to include at least tenfold more insulin-sensitive and insulin-resistant participants.

In conclusion, we have identified several novel mutations in the *IRS2* gene, which, despite no clear segregation with insulin resistance in this study, merit further investigation in additional cohorts; some variants (e.g. P829S) may yet be shown to have an effect on insulin sensitivity in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Table 1

Non-synonymous variants found in the *IRS2* gene of participants with severe insulin resistance (total SIR, n=161; Europid SIR, n=114) and Europid controls (n=173)

Participant	Phenotype	SNP	PolyPhen[a] / SIFT[b]	Genotype	Ethnicity	Fasting glucose (mmol/l)	Fasting insulin (pmol/l)	BMI (kg/m^2)
SIR1	SIR, pseudoacromegaly	c.233G>A p.S78N	Benign / Tolerated	GA	Asian	5.6	212	44.6
SIR2	SIR, pseudoacromegaly	c.1498C>G p.L500V	Benign / Tolerated	CG	Asian	6.0	264	39.7
SIR3	SIR short stature	c.1570A>G p.I524V	Benign / Affect protein function	AG	Turkish	4.9	1358	19
SIR4	SIR, pseudoacromegaly	c.2485C>T p.P829S	Benign / Tolerated	CT	Ashkenazy	5.1	507	25.5
SIR5	SIR	c.2566G>A p.A856T	Benign / Tolerated	GA	Europid	4.7	333	28
SIR6	SIR FPLD1	c.2834C>T p.S945F	Benign / Affect protein function	CT	Europid	5.5	155	obese
SIR7	SIR, pseudoacromegaly	c.3424G>C p.G1142R	Possibly damaging / Affect protein function	GC	Europid	8.5	880	47.3
SIR8	SIR, pseudoacromegaly	c.3983A>G p.H1328R	Possibly damaging / Affect protein function	AG	Europid	6.8	246	37.3
Control 1	Healthy volunteer	c.1379C>T p.P460L and c.3983A>G p.H1328R	Benign / Possibly damaging	CT	Europid	5.5	47	24.6
Control 2	Healthy volunteer	c.3400A>G p.K1134E	Benign / Affect protein function	AG	Europid	5.1	65	31.7
Control 3	Healthy volunteer	c.3788G>T p.G1263V	Probably damaging / Affect protein function	GT	Europid	6.4	43	24.3
Control 4	Healthy volunteer	c.3983A>G p.H1328R	Possibly damaging / Affect protein function	AG	Europid	4.9	45	23.4

FPLD1, familial partial lipodystrophy type 1, Online Mendelian Inheritance in Man (OMIM) (http://www.ncbi.nlm.nih.gov/omim) database ID: 608600; IR, insulin resistance

[a] PolyPhen predicts the possible impact of an amino acid substitution on the structure and function of a protein based on physical and comparative considerations

[b] SIFT predicts whether an amino acid substitution affects protein function based on sequence homology and the physical properties of amino acids